Lab Meeting
February 25, 2004

Introduction and Welcome

Dr. Eckfeldt began the meeting by reviewing the agenda. He noted that the main goal of meeting was to review the status of manuscript and aim to complete at least a draft version of the manuscript so it can be used as a handout at the manufacturer's forum in July. Dr Miller added that some of the redundancy in the manuscript needed removal.

Dr. Hostetter welcomed the group with a related poem. He mentioned that the *Suggestions for Laboratories* and *Rationales for GFR* documents went out to ASN members (5,500 qty.) last fall. NKF will also be sending the documents to their members (6,000+) in early March. Dr. Hostetter handed out a copy of a regulation that came out mandating reporting estimated GFR using serum creatinine (using Cockcroft-Gault) in France recently. The French doctor said it seems more people are being seen by nephrologists as a result of this law. Another group in Europe, EC4 (a consortium that's representative of societies of clinical chemists and pathologists), is concerned about the emerging changes occurring in manufacturers changing their calibrations.Their concern seems to be that estimate GFR results based on recalibrated creatinine methods could cause misinterpretations which could also result in changing medication based on wrong levels. Dr. Greenberg can pursue looking into this as it may be good to send a letter from the NKDEP Laboratory Working Group to let them know our mission.

Dr. Eckfeldt initiated introductions around the room (see participant list) and acknowledged other members that were not in attendance. Mauro Panteghini is new to the Working Group. He is a clinical pathologist in Italy and is involved with IFCC initiatives. Fred Van Lente is also new to the Working Group. He is a Biochemist at Cleveland Clinic and is directing labs that do creatinine measurements for various NIH research studies, including the MDRD study. He is also involved with a new NIH initiative to look at GFR estimating equations. The goals of this new program is to further refine the MDRD equation for other subsets (i.e. races, range of GFR, and diabetes etc) and to look at cystatin C to see how well it works for estimating GFR. It is also looking at the predictive value of urinary protein and re-measuring and analyzing from other clinical trials. The new initiative is just getting started and will go on for 4 years to refine and validate prediction equation. Andy Levey was also unable to attend the meeting and is a chief nephrologist at ??? in Boston and was involved in developing these equations.

The Fairview-University Experience with Estimated GFR

Dr. Eckfeldt presented data that was accumulated at the University of Minnesota, which sets the stage for the goals of the lab group. The study looked at if plasma serum differences impact the freezing comparison of various methods. Dr Michael Steffes accumulated most of the data. Initially, the group took at the impact of freezing and thawing serum, heparinized plasma, and EDTA plasma to be sure the freezing process had no impact on results. The study concluded that freezing did not appear to do anything to the samples.

Dr. Eckfeldt discussed results that looked at serum vs. plasma (refer to slide #4). Previous studies had shown that EDTA-plasma gave a slightly lower (3 to 5%) cholesterol values compare to simultaneously collected serum.. A later study looked across methods and discovered the magnitude of the discrepancy seems to vary by method. HDL is a larger effect actually higher values in EDTA-

plasma, likely due to a metal ion binding during the precipitation step. Dr. Hostetter asked how much higher is the protein in EDTA-plasma vs. serum? Dr. Eckfeldt responded by saying that 5% of plasma protein. Fibrinogen concentration, which is lost during clotting, is about 300/dl/mg with a total protein concentration of 6 to 7 g/dL.

In slide #5, the basic conclusion is EDTA-serum and Heparin gave comparable values. It was concluded that 1) freeze/thaw has little impact on measured creatinine, at least when not done repeatedly, 2) serum, EDTA-plasma, and heparinized plasma give comparable creatinine concentrations as measured by Jaffe methods, 3) when measured by enzymatic methods, there is a suggestion that EDTA-plasma yields slightly lower measured creatinine concentrations compared to simultaneously collected serum or heparinized-plasma. Dr. Miller inquired if anything has ever been published on the freeze/thaw study. Dr. Eckfeldt said he had not come across anything. Dr. Eckfeldt added that he and Dr. Steffes are going to try to publish this data.. Dr. Hostetter will ask Andy Levey and Joe Coresh about what information they might have on the impact of freezing samples.

The J&J Vitros vs. Beckman CX3 results show a lot of scattered plots between the range 1 and 2, which is particular critical. The CX3 was the MDRD equation in this comparison. The caveat of this comparison is CX3 only reports creatinine concentration to the tenth of a mg/dL, while the other instruments had been set to report two decimal places.

In the next slide, Dr. Eckfeldt showed the Roche/Hitachi vs. CX3. Dr. Greenberg though the 911 and Vitros used different coupled enzymic reactions.
On the 911 Jaffe vs. Roche enzymatichas better correlation than Roche enzymatic to Vitros enzymatic.

On the Roche/Hitachi Rate Blanked Jaffe slide, Dr. Eckfeldt noted the outlier points (three of them), which made them, think there was something erroneous about the iohexol clearance. They deleted these from some of the outliers from some of the testings.

Linear Regression analysis was first done with all the data (removing the three outliers) and then with the data points with GFR's greater than 100 by iohexol clearance removed. Correlation data sets aren't bad.

Dr. Eckfeldt summarize that, 1) EDTA plasma seems to give slightly lower values, but needs to be confirmed; 2) freezing serum has little impact on measured creatinine concentration; 3) Beckman CX3 gives on average estimates closest to the GFR measured by iohexol clearance; 4) there is not a simple or constant or proportional bias between methods; 5) when you put a measured creatinine in the MDRD equation, whether you're high or low, depends on the creatinine concentration; and 6) significant improvements need to be made accross the concentration range. The question may come up regarding which method is really correct. Eckfeldt mentioned that there may be a need for high level reference methods to look at individual patient samples to find what's going on. The LC MS will need to be used to find the real correlation. Dr. Hostetter asked how you select which method to use. Dr. Eckfeldt responded that all of them would need to be used and will need to be able to run dozens of samples per day.

Dr. Hostetter pointed out that NHANES tested samples using HPLC, which resulted in regression of the mean. Dr. Miller noted that when you pool large numbers of donors, you do average out non-specificity/anomalies, which becomes a powerful tool.

Dr. Eckfeldt reviewed his last slide and said he got great feedback from nephrologists but occasionally got hostile responses by primary care physicians. Initially, the group decided they would report numerical values under 80 and not 60. They found out 30% were being reported as less than 80. This would worry the patients and the doctors. Due to negative feedback, they changed it to 60. Dr. Greenberg raised the issue of what do insurance companies cover for further evaluation of creatinine-estimated GFR's. Dr. Hostetter noted that CMS now pays for consultation with a dietician for early kidney disease. Then it follows the guidelines put out by NKF, RPA, and JNC. For example, blood pressure over 130/80 should be prescribed and ACE/ARB. Dr Hostetter mentioned that the QI working group (sponsored by CMS) of the NKDEP may spark CMS to start tracking these as health care system performance measures.

Dr. Eckfeldt reminded the group that there are two goals the working group is faced with, 1) standardization of creatinine and 2) analytical specificity.

Dr. Eckfeldt also mentioned that pharmacist's dosage adjustments are based on the Cockcroft-Gault as opposed to the MDRD since it incorporates body size. The size of the dose is adjusted by body size. Drug half-life is influenced by GFR for renally excreted drugs, and estimated GFR is used mainly to alter the dosing interval. Another problem is that anyone's creatinine under 1.0, pharmacists rounded up to 1.0. As a result of fairly significant differences between MDRD and CG calculated GFR's, pharmacists may need to be engaged in the discussion. Dr. Eckfeldt has critical care pharmacist questions in a listserv that he can send out to the group.

Dr. Greenberg suggested that given the variation seen from method-to-method, the group should be extremely careful how we set the accuracy base for the reference system. Dr. Killeen asked if there is data on the accuracy of the iohexol measured GFR's and how they compare to iothalmad.

Status of CAP FFS Manuscript

Dr. Miller handed out the abstract submitted to AACC. He started explaining the method section of the study. The tables reflected data from a mix of responses at an 80% response rate. Dr Miller pointed out that the tables need to be updated for the final data set, which got a 98% response rate (5904 lab results). The CAP survey from October 2003 included a full fresh frozen serum prepared under NCCLS C37-A. This method collects individual blood, sits in ice bath to prevent clotting. Within 15 minutes, then plate cells are removed and the specimen is moved to room temp to clot. Then it was frozen at -70 degrees, saved 6-8 weeks, thawed, pooled, aliquoted, and refrozen again. They used high quality serum to represent a serum sample of fresh collected specimen from individual taken in laboratory. The value was .902 mg/dl HPLC creatinine that gave a slightly high creatinine. There were not enough n values to calculate mean. Dr. Miller mentioned that not many outliers were removed. With the exception of 2 methods, 1-3 responses from each method were removed. Five instrument manufacturers account for 97% participant results and 50 different method instrument peer groups were used. Dr. Miller added that working with a handful of instrument manufacturers can be very effective.

Dr. Miller's study found that if the instruments are grouped by method, the Roche platforms show smaller biases, but doesn't seem to matter what chemistry they're running. The LX Beckman instruments show less calibration biases and CX Beckman are much different. Typically, the instrument, race, and calibrator are not always from the same vendor. Peer grouping is used for the purpose of evaluating patients. It was inappropriate to conclude on Roches or Beckmans instrument. By in large, Hitachi has very little bias. CAP 2003 slide represents 95% of participant data so it is

pretty final. A program using these 5 methods will achieve harmonization we are trying to achieve. The peer groups are as they exsist in the CAP survey. A lot of these are putting themselves in the right category and CAP isn't able to sort them out. Dr. Killeen pointed out that the college uses previous data and when the manufacturer updates their instrument, CAP does not know.

Manuscript will be distributed to co-authors in a month and submitted by mid-April.

CAP Linearity Study

Dr. Eckfeldt explained that the CAP Linearity Survey and NIST reference material are on schedule and serum pools should be made in March. They are using the NCCLS protocol as Greg proposed made by Solomon Park. The materials will serve two purposes. First, they will serve as 2-level standard reference material for NIST. The native low pool is will used only female donors so it should be about 0.75-0.8 mg/dL. AR grade creatinine will be added to this low pool to make the high pool about 4.0 mg/dL.. Solomon Parke will make 1200 vials of each level of NIST. Dr. Eckfeldt has suggested Pat Clapshaw increase pool sizes by 10% for the laboratory working group. Additionally, NIST will not assign values for another 6 months. In addition to the intermix pools, they are going to make a low pool with buffered human albumin at 60% value of the the low native pool for pediatric range creatinine concentrations It was suggested that Paul Johnson at CAP's central office check with Elaine Staley at CAP or Pat Clapshaw to make sure the five major manufacturers of creatinine methods are represented in the linearity pilot. Paul can look at list and determine if appropriate.

Serum pools will be made available as a commercial CAP Linearity Survey product if it works in the pilot. As soon as the pilot program data is in, they will create new pools. Survey catalog advertising go out in fall 2004. Dr. Eckfeldt mentioned that they can look at actual performance not just peer group relationships. Dr. Killen said if there is a failed accuracy, we do not want to blame the participant. Therefore, we will need to look at some sort of dual grading method. It was asked if there is regression against true values.This study will enhance manufacturers and adjusting their calibrations. Dr. Miller mentioned that CAP needs to validate analytical range for the full measurement range of 20, this survey ends at 4 . Laboratories may elect not to purchase it. Marketing for the catalog needs to be thought through. They need to understand that this will help them with respect to NKDEP and practice of lab medicine better even though this survey will not help them meet their regulatory requirements.

Dr. Eckfeldt should talk with Susan Williams about the survey and how it's being introduced. Dr. Miller said to build in the fact that it is a product design to calculate the GFR and could possibly include their GFR equation results to verify their equation. Dr. Eckfeldt will try to talk to Susan to get a draft of the marketing piece/verbiage. Once the material is made, will NIST be willing to assign high and low pools? Dr. Eckfeldt suggested that Dr. Welch should begin thinking about this. It will be harder to market if no values are assigned. Mike thought LCMS method will most likely be in place. Dr. Eckfeldt only foresees needing the two values so there wouldn't be a huge amount of analytical work. Dr. Greenberg is a little concerned with how it will be received and what do we expect the labs and the manufacturers to do after the study is completed? Do we want the manufacturers to make these changes? And if so, is there a timetable for the changes? Dr. Eckfeldt pointed out that we should communicate this at the manufacturer's forum and in the manuscript. Would it be easier if there was a NIH mandated standardization? There needs to be total error specificity in GFR calculation. Dr. Myers said we can't make changes and step back. It will be a big educational effort outside the laboratories depending how far it's taken. Is the target IDMS? IDMS from different parts of the world give the same answer. However, this will affect the MDRD equation. The Chronic Kiedney Disease Epidemiology Collaboration (CKD-EPI) will likely not be ready for a year and the shift in

manufacturer calibration wouldn't take place until 2005. Dr. Eckfelct mentioned that *CAP Today* has an article coming out in March that will raise awareness about linearity survey, NKDEP, etc.–it has circulation of 25,000. Dr. Miller reminded group that cholesterol program took 5 years to get implemented. In order for manufacturers to change labels on instruments, etc. we will need 3-5 years.

Manufacturer's Forum

The goal for the manufacturer's forum is to introduce proposed timeline, identify potential steps, and get feedback. The forum will be July 26 from 10-12:00 at the AACC conference. The group suggested sending personal invitations to the manufacturers. Dr Greenberg offered to be the contact to use Avamed as an avenue to get the invitations out. Dr. Fleming can also help to get specific names within the industry as well as large reference labs. Product managers would be the audience we would need to contact. In addition to the Product Managers, we should also invite the top five LIS vendors (HBO, Mysis, Cerner, Meditech) as well as large reference labs. Dr. Greenberg can obtain this list. Dr. Hostetter offered that NKDEP could put together letterhead with the lab working group for the invitation letters. We also need to get in touch with Gail Mutnik for the room assignment for the forum. Dr. Killen is attending a meeting on March 17 involving LIS and will hand out the invitation at the meeting.

The group proposed the following agenda items for discussion at the manufacturer's forum:

- Introduction of why important (Hostetter)
- Brief summary of edutrack session on Fresh Frozen Serum (Miller)
- Synopsis of interlaboratory comparison data (Myers)
    - Identify potential goals/plan and timeline
- Current "State of the Art"/Available resources
    - linearity study, plots from linearity study, NIST reference system, calibration story and,
    - specificity issues (Killeen or Kaufman)
- Hand out manuscript draft
- Error budget and 30% total error in estimated GFR (Miller's slide CAP2003 C-C FFS)
- Question and Answer

Manufacturers will need to know where they can go for reference method analysis. NIST is unable provide that right now. Manufacturers need to be comfortable with the commutability of these materials. The best way to validate commutability is to do a high level reference method and field method analyses simultaneously on reference materials and clinical serum samples with creatinines in the same concentration range to see if it falls right on the line

Dr. Miller pointed out that we need to be careful with terminology as to "within-lab precision" vs. "between-lab precision". Currently "within-lab imprecision" is likely < 3% for most automated methods. Dr. Eckfeldt suggested getting help from statisticians to validate the above equation. Dr. Hostetter can look into this using Tom Green or Dr. Francis. . The underlying error will need to be pulled out of this equation. It may be beneficial to look at Joe Coresh's data. Dr. Miller pointed out trueness bias needs to be fairly small. Carl Vergo would be good to assist with this. It would be helpful to take this to the manufacturers meeting to explain the situation and get feedback.

Those attending the manufacturer's forum will be: Tim Larson, Tom Hostetter, Neil Greenberg, Gary Myers, Greg Miller, Harvey Kaufman, Anthony Killeen, and Micheal Welch. It would be a good idea to get it listed in the program booklet (both versions – the one sent prior to the conference and the one

distributed at the conference) and on the AACC website. Dr. Greenberg will handle placing it on the website. A description of the forum has been submitted to AACC for the program book.

Dr. Eckfeldt reviewed the memo distributed at the meeting that discussed the travel to the lab working group meetings. If any member has a problem not being able to attend AACC, please contact Tom.

Manuscript Section

Tab 8 includes the cholesterol program in 1988 to use as a reference. The group will be using this as a template for the manuscript. Tab 4 includes the manuscript that Dr. Myers revised. The group agreed that it's ideal if the members write everything in and people can react to it since it's a draft.

Under pre analytics, there needs to be pre-analytical biological factors that contribute or alter a normal creatinine level. Dr. Fleming can continue to work on this. Anything that's been done with respect to biological variability can be added to this section. Dr. Eckfeldt has this information and can write this section. Dr. Fleming can talk about bias and what's been done regarding reference methods.

The references of the manuscript should be formalized/standardized as footnotes on article. Elisa Gladstone can work on this.

Dr. Kaufman will write the section on the performance specifications for GFR and analytical and identify differences, variability, and bias.

Dr. Myers included interferences under each method. It was suggested to include interferences specific to assay types. Dr. Myers indicated that it flowed better as opposed to creating separate sections for interferences.

The group concluded that the introduction needs to incorporate the following messages: 1) a formalized acknowledgement mentioning that the article is based on serum creatinine as opposed to urine creatinine, 2) note the different methods for estimating GFR and how closely they come to each other, 3) identify what the more practical approaches of direct measurement of GFR (gold standard - more "silver"standard), and 4) MDRD is based on iothalmade and explain why that's good. Dr. Hostetter will come up with introduction.

The references should include NIST as well as others. It was suggested to get a copy from BIPM website. Dr. Welch and Willie May can look at this across metrology institutes.

Terminology like "to a national metrology institute (NMI)" instead of specifying NIST.
Dr. Myers suggested identifying JCTLM as a tool that's been put in place to look at reference methods and materials. Mike Welch can work on NMI's key comparisons. There should always be traceability back to the NMI. It may be nice to get another key comparison on the new reference materials to submit that there is comparability.

In the manuscript now, we mention most efficient standardization is to use reference materials that are assigned by reference of high order. When not possible, we suggested that manufacturers will have to provide direct comparisons with references of higher order.

External surveillance program

Dr. Eckfeldt posed the question what programs similar to CAP's are available in other countries? Dr. Myers only knows of the Canadian program by Dr. David Secum. Dr. Miller knows a study where they used fresh frozen materials compared to non-fresh frozen serum (David Secum or Dutch could be contacted). The group suggested asking Dr. Panteghini if he knows of any programs internationally. International Federation of Clinical Chemistry (IFCC) could have this - or EQAS per Dr. Myers. Dr. Myers could check with Joe on this to see if they provide this. Dr. Eckfeldt mentioned that if manufacturers are going to calibrate their instruments should know what they're being put up against. Dr. Kaufman suggested that this may be out of the scope of the manuscript, in that we're not trying to improve the EQAS around the world. An international measure Program 37 (IMP) used a frozen liquid material that was sent out to a large number which could be cited. Dr. Miller could send an email with questions to the providers. Dr. Myers will look for a meeting participant list from meeting related to EQAS providers. This could be added to complete the surveillance program providers.

National Resources will specifically cite what's available from NIST and CAP, unless there are labs in the U.S. with a traceability list as a second tier.

Another option is to say that labs that need to do fresh sample comparisons should seek out a qualified reference labs that can document their traceability back to NIST. Also, suggest that people go to JCLTM website to refer to the labs. NIST will have approved references and materials, but possibly not labs.

It was discussed that it may be necessary for a summary of recommendations for government, manufacturers, clinical lab, patient providers (i.e. green book for total cholesterol). Dr. Eckfeldt and Myers can work on this. In the green book, they put things that need to be done. Dr. Myers can send the federal document number of the Green book from NHLBI. Dr. Hostetter may also be able to obtain a copy.

Dr. Hostetter raised the concern of urinary albumin measurements and their accuracy. Dr. Eckfeldt said CAP will be offering a Linearity Survey for urinary albumin this year.

In summary:

- Submit revised sections to Gary Myers by April 1.
- Dr. Myers will revise the draft manuscript by April 15.
- The next call will be in May to discuss the revised manuscript. The call agenda will be:
    1) Finalize program for July AACC manufacturer's forum
    2) Discussion status of draft manuscript

Other important dates/timelines:

- Formulate questions and send to get biostats section completed by July meeting - Tom will contact Andy Levey first and then NIH person about the biostatistcs; division of background of the specifications.
- Finalize references (Elisa)
- Drs. Miller, Myers, Greenberg, Sam Caudill, Eckfeldt, and Hostetter will participate in conference call on comutability on Wednesday, March 10 at 3:00 pm EST.

Lab Working Group
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Attendees

John Eckfeldt, MD, PhD
Professor
University of Minnesota, MMC 609
Fairview-University Medical Center, Mayo Building, Room 763-1
420 Delaware Street SE
Minneapolis, MN 55455
eckfe001@umn.edu

James Fleming, PhD
Associate Vice President and Director of Scientific Affairs
Laboratory Corporation of America
112 Orange Drive
Elon College, NC 27244
fleminj@labcorp.com

Elisa Gladstone, MPH
Associate Director, NKDEP
National Institutes of Health
6707 Democracy Boulevard, 9th Floor
Bethesda, MD 20817
gladstoneE@extra.niddk.nih.gov

Neil Greenberg, PhD
Manager, Regulatory Affairs
Ortho Clinical Diagnostics
100 Indigo Creek Drive
Rochester, NY 14626-5101
ngreenbe@ocdus.jnj.com

Ethan Hausman, MD
Pediatrician/Pathologist
FDA
HFZ-440
2098 Gaither Road
Rockville, MD 20850
exh@cdrh.fda.gov

Thomas Hostetter, MD
Director, NKDEP
National Institutes of Health
6707 Democracy Boulevard, Room 622
Bethesda, MD 20817
hostettert@extra.niddk.nih.gov

Harvey Kaufman, MD
Medical Director, Hospital Services
Quest Diagnostics
1290 Wall Street West
Lyndhurst, NJ 07071
kaufmanh@questdiagnostics.com

Anthony Killeen, MD, PhD
Chair, CAP Chemistry Resource Committee
University of Minnesota
420 Deleware Street, SE
Minneapolis, MN 55455
kille001@umn.edu

Timothy Larson, MD
Mayo Clinic
200 First Street, SW
Rochester, MN 55905
larson.timothy@mayo.edu

Greg Miller, PhD
Consultant to CAP Chemistry Resource Committee
Virginia Commonwealth University
403 N. 13th Street
PO Box 980286, Room 501
Richmond, VA 23298
millerg@mail2.vcu.edu

Gary Myers, PhD
Centers for Disease Control and Prevention
4770 Buford Highway, NE
F25
Atlanta, GA 30341
glm1@cdc.gov

Michael Welch, PhD
Research Chemist
National Institute of Standards and Technology
Mailstop 8392
Bldg 227, A139
Gaithersburg, MD 20899
michael.welch@nist.gov